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Optimization of Solid State Fermentation of 2-Deoxy-D-Glucose *Aspergillus niger* Mutant for Improved Cellulase Production

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Abstract: Mutation and optimization of solid state fermentation of *Aspergillus niger* were performed in order to enhance the production of lignocellulolytic enzymes for biotransformation of cellulosic wastes. Spores of the fungus were irradiated with ultraviolet rays at 300µW/cm^2 at a distance of 15cm for 30 minutes. Mutants were selected in mineral salt medium containing 2-deoxy D-glucose (0.001 g/L). The selected *A. niger* mutant strain had a 2.1-fold increase in cellulase production more than the wild type. Solid-state fermentation of the selected strain was carried out and the optimal conditions for the production of cellulase were investigated. Optimum moisture for high enzyme yield was 20 %, temperature of 37°C, and a pH of 3.5. Other physical parameters included fermentation time of 4 days, inoculum size of 25% w/w and age of seeding culture of 4 days. When the medium was supplemented with corn-starch, glucose, lactose, sucrose and maltose; corn starch [9.21 U/mL and 2.30 U/mL Carboxylmethyl cellulose (CMCase) and total cellulose (FPase) respectively] enhanced the production of cellulase more than other substrates. Surfactants used were SDS, EDTA, Tween-20 and Tween-80. EDTA (9.39 U/mL and 2.30 U/mL CMCase and FPase respectively) enhanced enzyme production more than other surfactants. When $ZnSO_4$, $FeSO_4$, $CuSO_4$, $CaCl_2$, $MgCl_2$, $CoCl_2$ and KCl were used; $FeSO_4$ (9.29 U/mL and 2.46 U/mL CMCase and FPase respectively) supported cellulase production over others. Urea (9.52 U/mL and 2.37 U/mL CMCase and FPase respectively) was the best nitrogen supplement compared to malt extract, yeast extract, NH_4NO_3 and NH_4Cl. In all cases, there was an increase in CMCase and FPase activities. Optimization of the solid state fermentation of mutant resulted in a 7.4-fold and 7.6-fold higher yield in CMCase and FPase respectively more than the wild strain. This signifies its potential for use in the enhanced production of enzymes relevant in cellulosic wastes biotransformation.

KEYWORDS: *Aspergillus niger*, Cellulase, UV mutation, Optimization, Fermentation

1.0 Introduction

The world has realized the need to source for alternative fossil fuel. This is due to the impending threat of extinction of world crude oil reserve due to the ever rising consumption of fossil fuels. It has been estimated that by the year 2050, the world's crude oil production would decline five times below its current level (Mehdi *et al*., 2009). Ethanol has been employed to blend fossil fuel in varying proportions (Sun and Cheng, 2002). Ethanol can be easily manufactured from carbohydrate sources. However, the manufacture of ethanol from these sources creates a high competition with food. This inevitably led to the shift of attention towards the utilization of lignocellulosics.

Lignocellulosic wastes can be utilized not only for ethanol production but industrial chemicals. Howard *et al* (2003) reported that many organic chemicals can be produced from ethylene, propylene, benzene, toluene and xylene. Benzene, toluene and xylene can be obtained from lignin, being aromatic while ethylene and propylene can be obtained from ethanol derived from fermentation of glucose obtained from cellulose biodegradation.

Biodegradation of lignocellulosics can be achieved with the use of ligninocellulolytic enzymes produced by bacteria and fungi (Kaur

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et al., 2007). The performance of these microbes can be improved by different methods of mutation (Parek *et al*., 2000; De Nicolas Santiago *et al*., 2006; Vu *et al*., 2009; Pradeep *et al*., 2012). On the other hand, enhanced enzyme production can also be achieved by supplementations with salts (Junior *et al*., 2009), metals (Nikolic *et al*., 2009) and optimizing fermentation conditions (Archaya *et al*., 2008). Modification of microbes for enhanced performance by mutation and optimization of their fermentation conditions are the major approaches for enhanced ligninocellulolytic enzymes production, although, usually done independently.

In previous work, we tried to establish a suitable condition for mutating fungal spores with ultraviolet irradiation *vis-à-vis* the distance and duration of delivering irradiation. In this work, we aim to enhance cellulase production by mutating the fungi using UV rays, then optimizing its solid state fermentation conditions for enhanced performance.

2.0 Materials and Methods

2.1 Materials

2.1.1 Chemicals

All chemicals and reagents (analytical grade) used were obtained from Fluka, Germany. The Potato Dextrose Agar and Sabouraud Dextrose Agar were obtained from Biomark Laboratories, Pune, India, while yeast extract used was from Oxoid, England.

2.1.2 Cellulosic Materials

Aboria sawdust was collected from Okobaba sawmill, Ebute-Metta Lagos (N06° 28. 714' E003° 23. 426').

2.2 Methods

2.2.1 Pre-treatment of Cellulosic Materials

Aboria sawdust was pulverized, sieved through mesh of 0.250 mm to make uniform particle size. The powder obtained was pretreated by ammonia steeping according to modified method of Abu *et al* (2002).

2.2.2 Isolation and Maintenance of A. niger

A. niger was isolated from decomposing wood wastes obtained from Okobaba sawmills, Ebute-Metta, Lagos, Nigeria, using different selective and differential media. It was maintained on Potato Dextrose Agar (PDA) and modifed Czapek Dox Media at 25 ± 1°C. The fungus was identified from its cultural and morphological characteristics at the Department of Microbiology, University of Lagos, Nigeria. It was also genotyped to confirm its identity by sequencing its amplified ITS1 and ITS4 regions on its DNA.

2.2.3 Macroscopic and Microscopic Studies

Macroscopic study was done by examining the growth rate, texture, colour, and topography of the colony using PDA, yeast extract agar and Czapek Dox Agar. Microscopic study was done by preparing slide mount with lacto phenol cotton blue stain and observed under the light microscope.

2.2.4 Mutagenesis

The mutagenesis study involved finding the exposure time at which 50% of the *A. niger* were inactivated by ultraviolet radiation. Spores were inoculated in Petri dishes containing PDA at 30°C for 4 days. Spores were harvested, counted in Neubauer chamber and their concentration adjusted to $1x10^7$ spores per mL. The spore suspension (5 ml) was poured into a sterile Petri dish under agitation using a magnetic bar, during the whole procedure. The ultraviolet radiation source was placed at a distance of 15 cm above the surface of the suspension spores; radiation intensity was delivered at 300 $\mu W/cm^2$ (Montiel-Gonzalez *et al*., 2004), for 30 minutes. The spore suspension were placed on ice bath for 5 minutes, kept in the dark for another 30 minutes (to avoid photo reactivation), and diluted to 10^4 spores/mL.

2.2.5 Selection of Hyperproducing Mutants

The mutated *A. niger* strain was inoculated (0.5 mL) in Petri dishes containing PDA medium (0.3 g Urea, 1.4 g $(NH_4)_2SO_4$, 2.0 g KH_2PO_4, 0.3 g $CaCl_2$, 0.3 g $MgSO_4.7H_2O$, 1.0 g protease peptone, 5.0 mg $FeSO_4.7H_2O$, 1.6 mg $MnSO_4.7H_2O$, 1.4 mg $ZnSO_4.7H_2O$, 2.0 mg $CoCl_2$ and 0.001 g 2-deoxy-D-glucose). The pH of the media was adjusted to 5.0 ± 0.2, incubated at 30°C for 72 hours. The spores were washed with 10% saline over filter paper and dried.

2.2.6 Solid State Fermentation (SSF)

A 500 ml Erlenmeyer flask containing 20 g of pre-treated sawdust was added to minimal salt media (supplemented with 5 g glucose) at 40 % (w/v) and was sterilized at standard autoclaving conditions. This was inoculated with 5 ml of spore suspension of the mutated fungi previously adjusted to 1 x 10^6 spores per mL and incubated at 37°C for 3 days.

2.2.7 Preparation of Crude Cellulose

To the moldy set up, 50 mL of distilled water was added. The mixture was shaken in an orbital shaker at 200 rpm for 1 hour. This was later centrifuged at 5,000rpm for 10 minutes. The supernatant obtained was used as crude enzyme source.

2.2.8 Determination of Cellulase Activity

Enzyme assays were carried out according to the methods described by Singh *et al* (2009). Endoglucanase (CMCase) activity was determined at 40°C by using carboxy-methyl cellulose as substrate. For total cellulase/filter paper (FPase) activity, Whatman No.1 filter paper strip of dimension 1.0 x 6 cm (50mg) was placed in each assay tube. The filter paper strip was saturated with 1.0 ml of Na-Citrate buffer (0.05M, pH 4.8) and was heated for 10 minutes at 50°C. Half milliliter of an appropriately diluted enzyme in Na-citrate buffer (0.05M; pH 4.8) was added to the tube and incubated at 50°C for 60 minutes. The activity of cellulase was done using the procedure described by Singh *et al* (2009).

2.2.9 Optimization of Solid State Fermentation

The effects of various physicochemical parameters required maximal production of cellulase by the mutant *A. niger* was investigated using solid state fermentation. The parameters optimized were moisture (10-60% v/w), incubation temperature (25-50°C), pH (3-6), fermentation time (1-8 days), size of inoculum (%, w/w), and age of culture medium (days). Studies were also done to investigate the effect of cellulase production on various additives supplementation into the culture. The supplements investigated include carbon source (glucose, lactose, sucrose, maltose and corn starch), surfactants (sodium dodecyl sulfate SDS and ethylene diamine tetra acetic acid, EDTA, Tween-20, Tween-80), metal salts ($ZnSO_4$, $FeSO_4$, $CuSO_4$, $CaCl_2$, $MgCl_2$, $CoCl_2$ and KCl), and nitrogen additives (malt extract, yeast extract, NH_4NO_3, NH_4Cl and Urea).

2.2.10 Statistical Analysis

All experiments were carried out in triplicates, unless otherwise stated. The results were expressed as mean ± standard error of mean. The treatment means were tested for significant difference and compared with the control using the Dunnet interval test. The Student's t-test was used to compare between two groups. One-way analysis of variance (ANOVA) with Tukey's test was used to compare the level of significant difference between samples that were more than two; $p < 0.05$ was considered to be significant. These were done using the GraphPad Prism 5 statistical package.

3.0 Results

The colony of the wild fungi (Figure1A) with characteristic dense black appearance was reduced upon irradiation (Figure 1B). The selected mutant had a carboxymethyl cellulose activity (CMCase) of 4.69 U/mL as against the wild which was 2.24 U/mL (Figure 2A). This represented a 2.1 fold increase. Similar trend was found with total cellulase activity (FPase). The wild *A. niger* specie had a total cellulase activity of 0.86 U/mL while 2-deoxy-D-Glucose mutant had a total cellulase activity (FPase) of

1.97 U/mL (Figure 2B). Moisture content less than 20% was optimum for CMCase production using *A. niger* mutant. There was a sharp reduction in endoglucanase activity when moisture level was higher than 20%. The suitable moisture for optimum total cellulase production was 30 % (Figure 3A).

Temperature for optimum enzyme production was found to be 37 ^{o}C with endoglucanase activity of 9.33 U/mL and total cellulase activity of 2.12 U/mL (Figure 3B). As shown in Figure 3C, pH of 3.5 was found to be optimum for endoglucanase production (9.21 U/mL). Similar trend was observed for total cellulase production where a maximal enzyme activity of 2.16 U/mL was obtained at the same pH (Figure 3C). Fermentation time of 4 days was found to be optimum for CMCase and FPase production. Enzyme activities of 8.32 U/mL and 2.13 U/mL were observed for both classes of cellulase (Figure 3D).

Age of culture media suitable for optimum endoglucanase production was found to be 3 days with endoglucanase activity of 7.58 U/mL, while 4 days (2.26 U/mL) was found to be optimum for total cellulase activity (Figure 3E). An inoculum size of 20 % v/w (9.45U/mL) was suitable for optimum endoglucnase activity. However, total cellulase activity of 20 % v/w (2.26 U/mL) was found to be optimum for FPase (Figure 3F).

Corn starch was found to enhance the production of cellulase more than glucose, lactose, sucrose and maltose with a CMCase activity of 9.21 U/mL and FPase of 2.30U/mL (Figure 4A). EDTA enhanced more enzyme production than SDS, Tween-20 and Tween-80 with a CMCase of 9.39U/mL which was higher than the control (8.36U/mL) and FPase of 2.30U/mL which was higher than the control (2.19U/mL) (Figure 4B). $FeSO_4$, enhanced cellulase production more than $ZnSO_4$, $CuSO_4$, $CaCl_2$, $MgCl_2$, $CoCl_2$ and KCl with a CMCase of 9.29U/mL which was higher than the control (8. 36U/mL) and FPase of 2.46U/mL which was quite higher than 1.92 U/mL, the control (Figure 4C). Urea displayed the highest nitrogen supplement compared to malt extract, yeast extract, NH_4NO_3 and NH_4Cl (Figure 4D). A CMCase of 9.52 U/mL which was higher than the control (8.01 U/mL) while an FPase of 2.37 U/mL against the control (1.84 U/mL) was recorded. Malt extract and yeast extract had no significant effect on cellulase production. The parameters identified to be optimum for cellulase production was used to produce cellulase from sawdust using *A. niger*. Engoglucanase (CMCase) production was 16.55 U/mL after 72hr (Figure 5). This was 7.4-fold higher than the wild. The FPase activity was 6.54 U/mL (Figure 5) which was 7.6-fold higher than the wild.

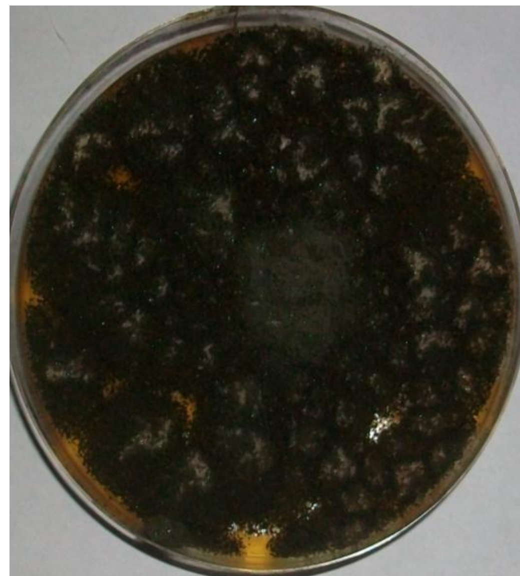

Figure 1A: *A. niger* wild strains grown on PDA.

Figure 1B: *A. niger* mutant strains grown on PDA

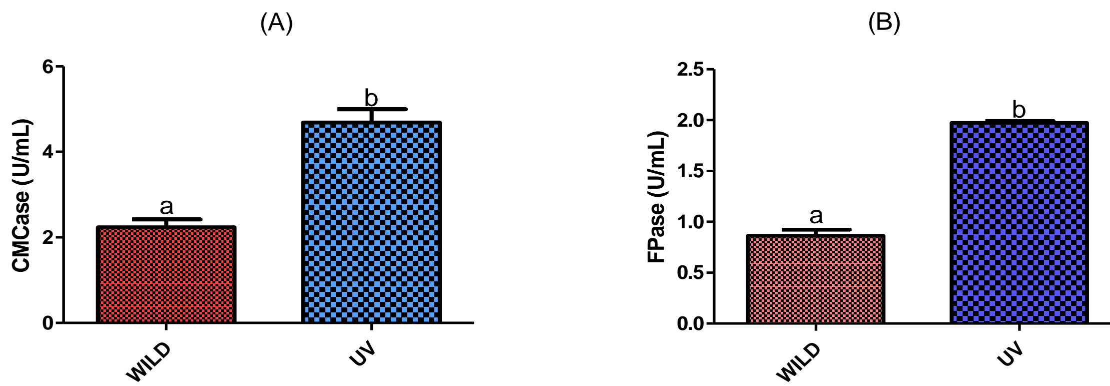

Figure 2: CMCase (A) and FPase (B) production by wild and UV mutated strain of *A. niger*
Bars with different letters imply that there was significant difference ($p < 0.05$).

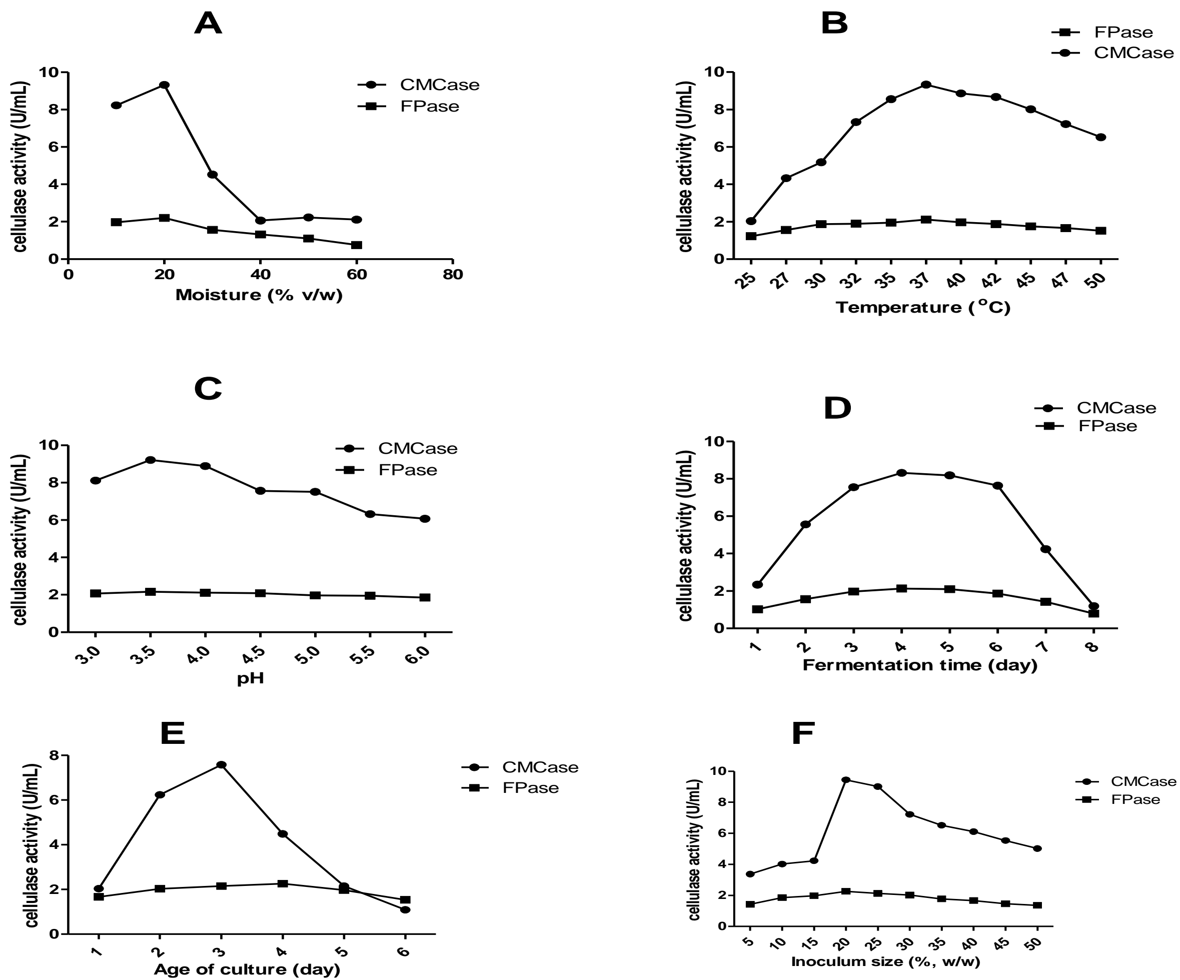

Figure 3: Effect of various culture conditions on cellulase production by mutant *A. niger*. (A) % Moisture; (B) Incubation temperature; (C) pH; (D) Fermentation time; (E) Age of culture (F) Inoculum size.

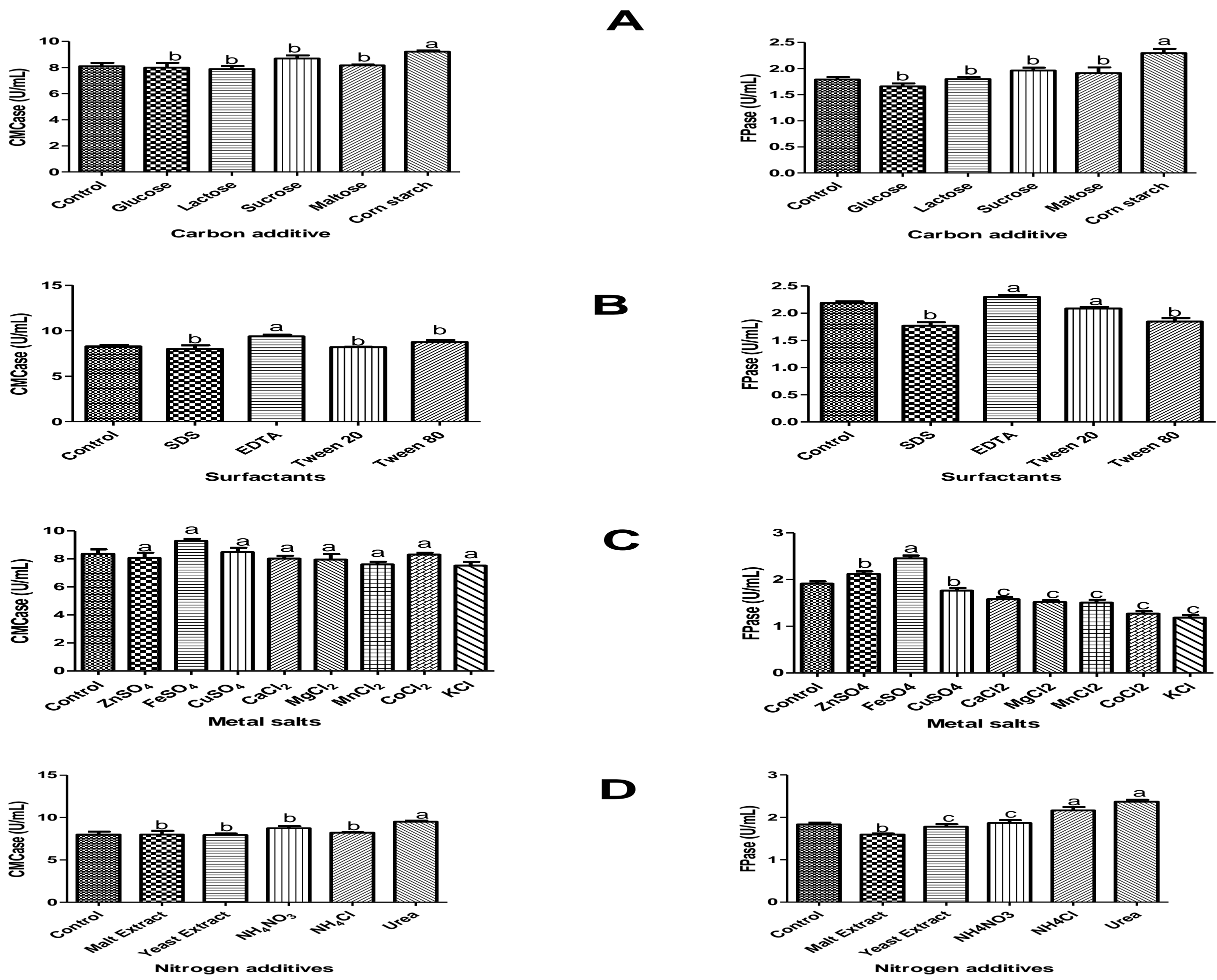

Figure 4: Effect of different medium additives on cellulase production by *A. niger* mutant
Bars with similar letters imply that there was no significant difference ($p > 0.05$).

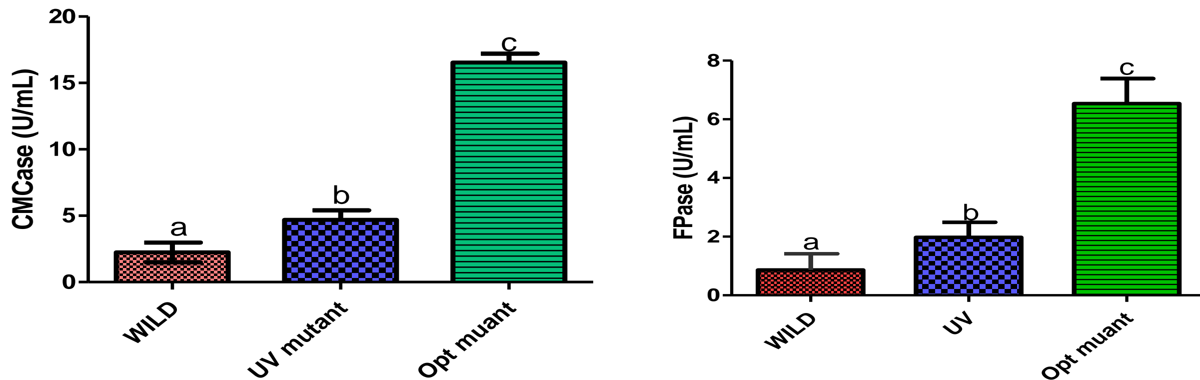

Figure 5: Cellulase activity from the wild *A. niger* strain, its UV mutant and the optimized UV mutant.
Bars with different letters connotes that there was significant difference ($p < 0.05$).

4.0 Discussion

Microbes, most especially fungi, posses the ability of producing a wide range of industrially important enzymes, which find useful applications in almost all industries including food, feed, textile, cosmetics and pharmaceuticals. These enzymes may however, not be available in commercial quantities. Hence, the need to modify these organisms for enhanced enzyme production.

The decrease in conidiospores of *A. niger* mutant growing on mineral salt agar containing 2-deoxy glucose might be due to the cytotoxicity of the ultraviolet radiation as well as the presence of 2-deoxy glucose in the culture media. The glucose derivative is an anti-metabolite which could cause catabolite repression to non-mutant strains (Farkas *et al*., 1981; Anwar *et al*., 1996). Only mutated strains of fungi can survive this repression (Dillion *et al*., 2005).

The increase in cellulase activity was a confirmation of mutation in the genome of the organism. UV irradiation could lead to unspecific mutation which may be beneficial or not. In this case, it enhanced the production of cellulase. Moisture is very essential for the production of cellulase. It is pertinent that moisture level should be at a specific quantity required for optimum enzyme production. Too much moisture in a solid-state fermentation medium could affect porosity of substrate (Vu *et al*., 2010a). The optimum moisture content obtained is comparable to the findings obtained by Vu *et al* (2010a) where moisture content higher than 50% v/w (76.6 U/g) was said to be optimum for mutated *A. niger*.

The optimum temperature of 37°C was found suitable for both CMCase and FPase. The optimum temperature obtained agrees with the findings from earlier studies of Narasimha *et al* (2006). The surfactant that best enhance cellulase production was EDTA. A pH of 3.5 was found to be optimum for enzyme production. There was a slight difference to the findings of Archaya *et al* (2008). They identified an optimum pH of 4.0 and 4.5 for wild *A. niger* species. The difference could be due to change in the genetic constituent of the organism due to its mutation by UV. Vu *et al* (2010b) obtained 28.5U/g for an optimum pH of 3.4.

Solid State Fermentation has been demonstrated to provide better hydrolytic enzyme that eventually yield more than the submerged. It is fermentation in the absence or near absence of free water with inert natural substrates as solid support (Pandey *et al*., 1999). During solid state fermentation, the components of the cell wall are degraded, and enzymes which hydrolyze lignocelluloses are produced and act optimally. Additional carbon source is needed in the fermentation tank to enhance growth. It is expected that the supplemented carbon source would be easily metabolized (Zhu *et al*., 2011). In this study, corn starch enhanced production of endoglucanase (CMCase) and total cellulase (FPase) activities more than glucose. This contrast the findings of Zhu *et al* (2011) where supplementation of glucose and increased moisture content enhanced endoglucanase production with *Trametes versicolor* during SSF of corn stover. It could be explained that the metabolic pattern of the organism may have been altered as a result of the mutation, thus making it to easily metabolise glucose biopolymers without hindrance.

Surfactants have been used to enhance lignocellulolytic hydrolysis; when used, they reduce enzyme loadings. A number of mechanisms have been proposed for their action. Ballesteros *et al* (1998) reported that Tween-80 bring cellulase in close proximity with cellulose. EDTA and Tween-80 enhanced enzyme activity of *A. niger* mutant. The enhancement could be due to the ability of the surfactants to reduce unnecessary attachment of cellulase to substrates other than cellulose in the medium, thus enhancing its performance. Yang and Wyman (2006) explained that bovine serum albumin, a biosurfactant, could attach to lignin, thus allowing non-specific attachment of cellulase to lignin when it is used in the hydrolysis of lignocellulosics.

Metal salts play significant roles in microbial metabolism. They act as cofactors or as co-enzymes. Some fermentation will not yield product without the inclusion of metal salts. $FeSO_4$ best enhanced the production of endoglucanase and total cellulase.

Urea supplementation enhanced enzyme activity more than other nitrogen supplements. This corroborates the findings of Archaya *et al*

(2008). Optimization of nitrogen source for cellulase production was carried out on sawdust using wild *A. niger* and urea was found to enhance optimum CMCase production more than peptone and sodium nitrite.

In conclusion, optimization of the solid state fermentation of *A. niger* mutant selected using 2-deoxy-G-glucose resulted in a 7.4-fold and 7.6-fold higher yield in CMCase and FPase respectively, more than the wild strain. This signifies its potential for use in the enhanced production of enzymes relevant in cellulosic wastes biotransformation.

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References

Abu, E. A., Ameh, D. A., Onyenekwe, P. C., Agbaji, A. S., Ado, S. A. and Nwaeze, A. R. (2002). Comparative studies of the effect of pretreatments of three Agrowastes on cellulose production by *Aspergillus niger* SL1. Scientia Africana 15(1): 57-63.

Archaya, P. B., Achaya, D. K. and Modi, H. A. (2008). Optimiztion or cellulase production by *Aspergillus niger* using saw dust as substrate. African Journal of Biotechnology 7(22): 4147-4152.

Anwar, M. N., Suto, M. and Tomida, F. (1996). Isolation of mutants of *Penicillium urpurogenum* resistant to catabolite repression. Applied Microbiology and Biotechnology 45: 684-687.

Ballesteros, I., Oliva, J. M., Carrasco, J., Cabanas, A., Navarro, A. A. and Ballesteros, M. (1998). Effect of surfactants and zeolites on simultaneous saccharification and fermentation of steam-exploded poplar biomass to ethanol. Applied Biochemistry and Biotechnology 70: 369-381.

De Nicolas-Santiago, S., Regalado-Gonzalez, C., Garcia-Almendarez, B., Fernandez, F. J., Tellez-Jurado, A. and Huerta-Ochoa, S. A. (2006). Physiological, morphological, and mannanase production studies on *Aspergillus niger* uam-gs 1 mutants. Electronic Journal of Biotechnology 9(1): 50-60.

Dillion, A. J. P., Zorgi, C., Camassola, M., and Henriques, J. A. P. (2005). Use of 2-deoxyglucose in liquid media for the selection of mutant strains of *Penicillium echinulatum* producing increased cellulase and β-glucosidase activities. Applied Microbiology and Biotechnology 70:740-746.

Farkas, V., Labudova, I., Bauers, S. and Ferenczy, L. (1981). Preparation of mutants of *Trichoderma viride* with increased production of cellulase. Folia Microbiology 26:105-110.

Howard, R. L., Abotsi, E., Jansen Van Rensburg, E. L. and Howard, S. (2003). Lignocellulosic Biotechnology: issues of bioconveion and enzyme production. African Journal of Biotechnology 2(12): 602-619.

Junior, M. M., Batistote, M., Cilli, E. M. and Ernandes, J. R. (2009). Sucrose fermentation by Brazillian ethanol production yeast in media containing structural complex nitrogen sources. Journal of the Institute of brewing 115: 191-197.

Kaur, J., Chandha, B. S., Kumar, B. A. Kaur, G. S. and Saini, H. S. (2007). Purification and characterization of β-glucosidase from Melanocarpus Sp. MTCC3922. Electronic Journal of Biotechnology 10: 260-270.

Mehdi, D., Heidi, S. and Wensheng, Q. (2009). Fungal bioconversion of lignocellulosic residues; Opporunities and Perspectives. International Journal of Biological Sciences 5(6): 578-595.

Montiel-Gonzalez, A. M., Viniegra-Gonzalez, G, Fernandez, F. J. and Leora, O. (2004). Effect of water activity on invertase production in solid state fermentation by improved diploid strains of *Aspergillus niger*. Process Biochemistry 39(12): 2085-2090.

Narasimha, G., Sridevi, A., Viswanath, B., Chandra, M. S. and Reddy, R. B. (2006). Nutrient effects on production of cellulolytic enzymes by *Aspergillus niger*. African Journal of Biotechnology 5(5): 472-476

Nikolic, S., Mojovic, L., Pejin, D., Rakin, M. and Vucurovic, V. (2009). Improvement of ethanol fermentation of corn Semolina hydrolyzates with immobilized yeast by medium supplementation. Food Technology and Biotechnology 47: 83-89.

Pandey, A., Selvakumar, P., Soccol, C. R., and Nigam, P. (1999). Solid state fermentation for the production of industrial enzymes. Current Science 77: 149-162.

Parekh, S., Vinci, V. A. and Strobel, R. J. (2000). Improvement of microbial strains and fermentation processes. Applied Microbiology and Biotechnology 54(3): 287-301.

Pradeep, M. R., Janardhan, A., Kumar, A. P. and Narasimha, G. (2012). Induction of chemical mutations in *Aspergillus niger* to enhance cellulase production. International Journal of Environmental Biology 2(3): 129-132.

Singh, A., Singh, N. and Bishnoi, N. R. (2009). Production of cellulases by *Aspergillus heteromorphous* from wheat straw under submerged fermentation. International Journal of Environmental Science and Engineering 1(1):23-26.

Sun Y. and Cheng, J. (2002). Hydrolysis of lignocellulosic material from ethanol production: A review. Bioresource Technology 83: 1-11.

Vu, V. H., Pham, T. A. and Kim, K. (2009). Fungal strain improvement for cellulase production using repeated and sequential mutagenesis. Mycobiology 37(4): 267-271.

Vu, V. H., Pham, T. A. and Kim, K. (2010a). Improvement of fungal cellulase production by mutation and optimization of Solid State Fermentation. Mycobiology 39(1): 20-25.

Vu, V. H., Pham, T. A. and Kim, K. (2010b) Improvement of fungal strain by repeated and sequential mutagenesis and optimization of solid-state fermentation for the hyper-production of raw starch digesting enzyme. Journal of Microbiology and Biotechnology 20: 718-726.

Yang, B. and Wyman, C. E. (2006). BSA treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates Biotechnology and Bioengineering 94(4):611-617.

Zhu, H., Zhang, H., Zhang, Y. and Huang F. (2011). Lignocellulose degradation, enzyme production and protein enrichment by *Trametes versicolor* during solid-state fermentation of corn stover. African Journal of Biotechnology 10(45): 9182-9192.